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Generation of a transgenic ORFeome library in *Drosophila*

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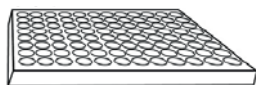
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Vector Preparation
&
Gateway ORF
Cloning



Barcode the *pGW-HA.attB* vector



Clone and sequence ORFs
into *pDONR221*



Transfer ORFs into
pGW-HA.attB



Determine barcodes of
pGW-HA.attB plasmids



Injections
&
Generating
ORF Strains



Inject pooled plasmids



Establish fly strains



Identify strains via barcode
determination



Confirm ORFs by single-fly PCR

Figure 1

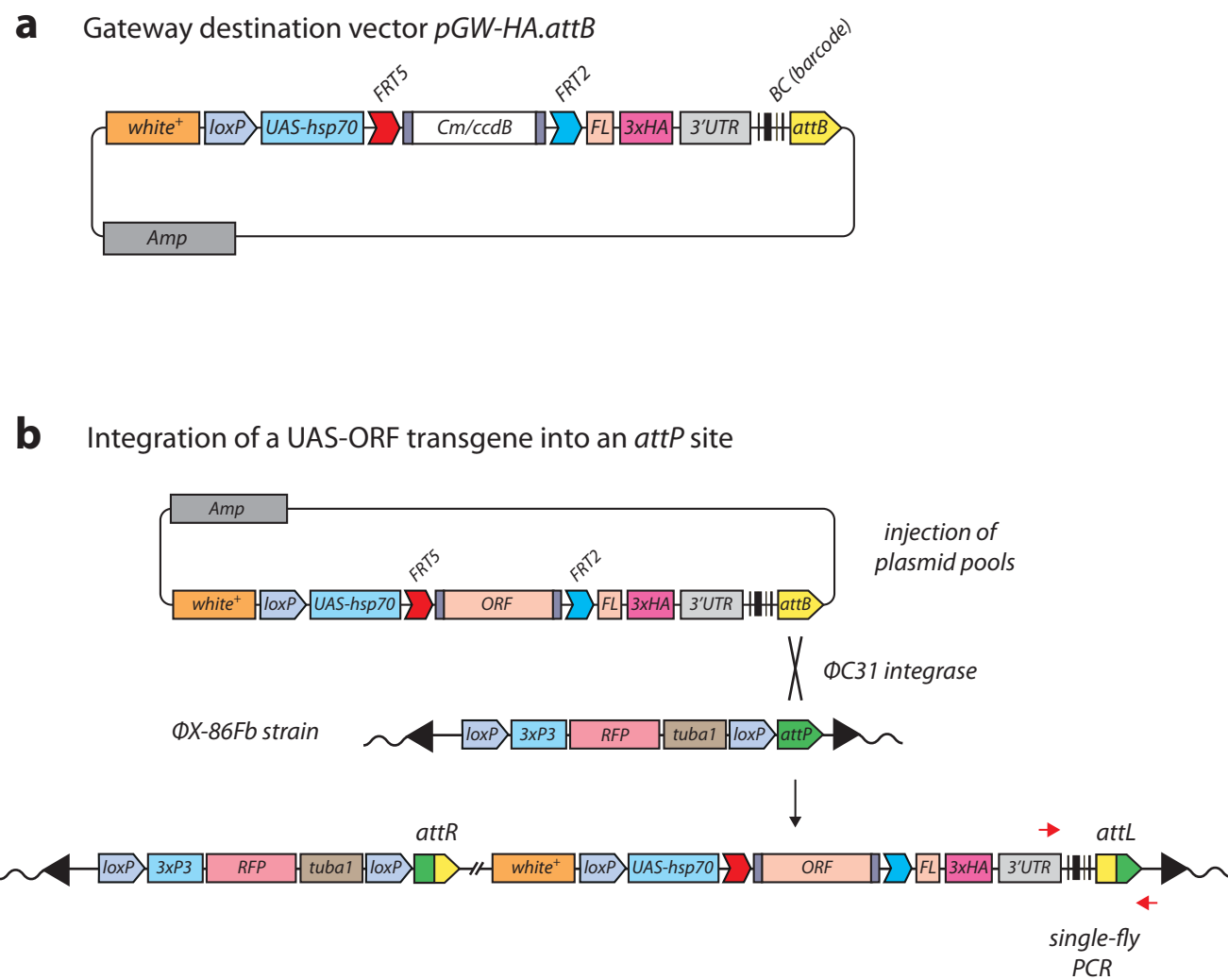


Figure 2

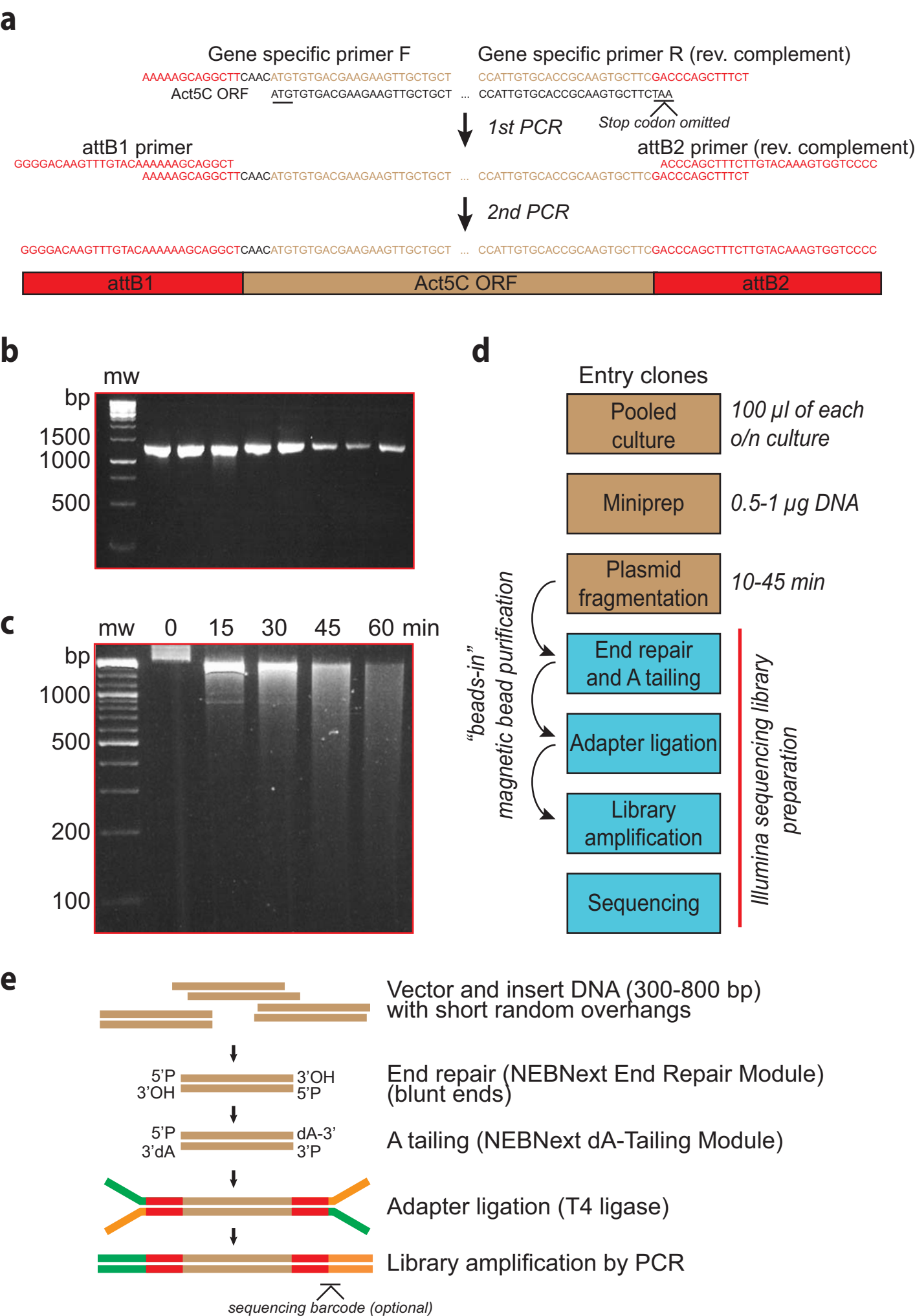
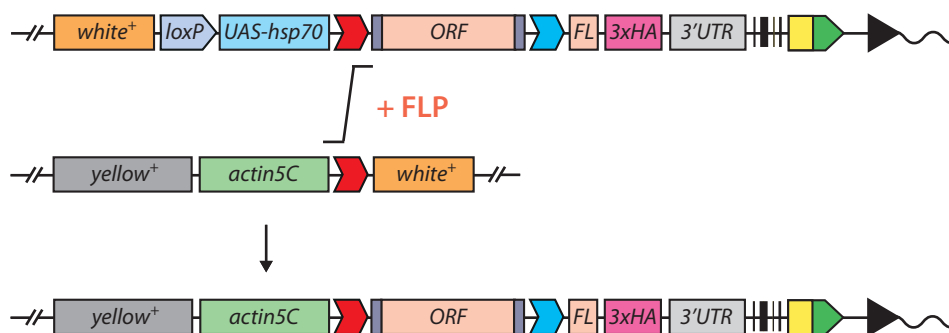
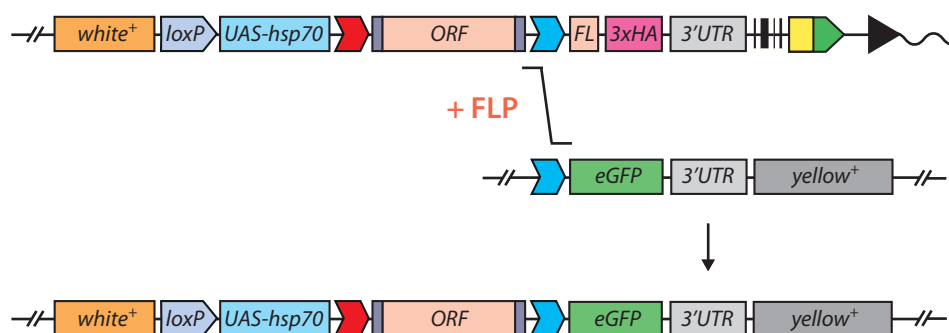
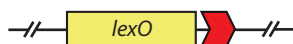
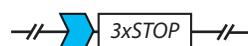
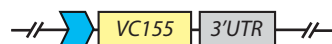
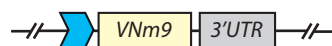


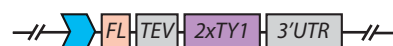
Figure 3

aN-terminal *in vivo* exchange**b**C-terminal *in vivo* exchange**c**

Additional N- and C-terminal swapping lines

Exchange to *lexO* promoterElimination of *3xHA* tag

Bimolecular fluorescence complementation

Exchange to *2xTY1* tag**Figure 4**

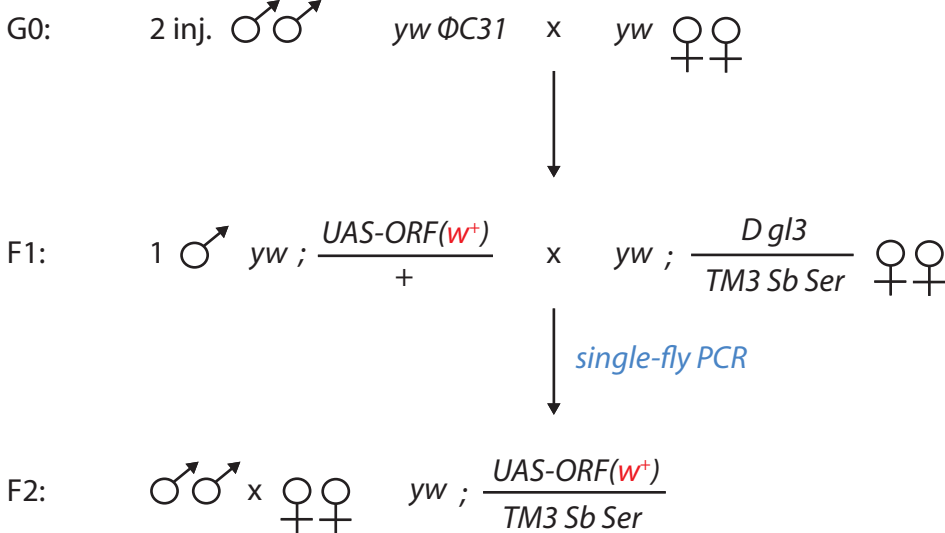


Figure 5